COLD INJURY -- FROSTBITE

THE PROBLEM:

There is no good way to treat a frostbitten extremity. At best, the physican currently can only avoid additional damage by overly vigorous treatment. In civilian practice the problem is of limited importance occurring primarily in those exposed to cold while intoxicated or following accidental outdoor exposure. But during wars, life and limb destruction by cold may equal or exceed casualties from enemy action. The names of campaigns serves to recall such terrible scenarios: The 1812 retreat from Moscow; Trench foot in World War I, Frostbite during battles in the Ardennes or Stalingrad in World War II; Chosin Reservoir in Korea.

The medical problem is two fold: 1) How to minimize tissue damage in a still cold extremity and, 2) How to treat an extremity following rewarming. The current studies only address the first question.

THE HYPOTHESIS:

It is suggested that a major factor in cold injury is due to the inflammatory response that occurs during and after rewarming and that this may be due to damage by leukokines released at the site of cold injury. If so, damage could be minimized by agents that would alter the inflammatory response before or during rewarming. The problem is analogous to reperfusion injury of the cold heart.

There are two schools of thoughts concerning the pathophysiology of frostbite. One holds that cold itself destroys cells; the other that the damage occurs during rewarming.

Mechanisms by which cold might damage cells include ice crystal formation with cytolysis due to expansion when water freezes, or by dehydration and hyperosmolarity secondary to intracellular water sequestration and local

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University of Colorado Health Sciences Center

University Hospitals
School of Medicine
School of Nursing
School of Dentistry

4200 East Ninth Avenue
Denver, Colorado 80262

November 11, 1982

Commander Douglas Strong
Medical Service Corp
Naval Medical R & D Command
National Naval Medical Center
Bethesda, Maryland 20014

Dear Commander Strong:

I enclose an Annual Report of our Cold Injury--Frostbite grant from the Navy No. N00014-81-K0731. This includes our requested budget for the next year.

A few days ago, I sent an informal letter as per your request summarizing this report. We are extremely enthusiastic. Our original hypothesis that cold injury can be blocked pharmacologically using newer knowledge of the mechanisms and immunologic response and familiarity that prostaglandin and prostaglandin inhibitors, as far as I know, is a completely new approach to this problem.

I greatly appreciate the support you have shown us and hope that someone can visit our laboratory and discuss the problem with us in the near future.

Sincerely,

Ben Eiseman, M.D.
Professor of Surgery
Department of Surgery

BE:jn
Encl.

cc: Mr. G. Max Irving, (1)
Administrative Contracting Officer

Director, Naval Research Laboratory (6)
ATTN: Code 2627
Washington, D.C. 20375

Defense Technical Information Center (12)
Bldg. 5, Cameron Station
Alexandria, Virginia 22314

hyperosmolarity following ice crystal formation. There is no histologic evidence of damage by cold as any examination of a frozen section specimum will attest. Microscopic evidence of injury occurs during and after rewarming.

Physiologic changes following cold exposure have been extensively summarized and need not be repeated. Aggregation of platelets and leukocytes in the cold tissue has been noted as a part of the process but emphasized in the classic literature only as they may cause vascular plugging. The recent increase in knowledge concerning the inflammatory and immune response suggests that lymphokines and toxic substances from platelets might be operative in frostbite injury. Frostbite must be re-examined in light of recent knowledge of the immune and inflammatory response.

TREATMENT:

A wide variety of techniques have been examined clinically and experimentally in the treatment of cold injury but none has been reliably effective in blocking such tissue damage. The frustrating part of the problem is that tissue damage seems to occur under the eyes of the physician as he rewarms the extremity.

Suggested methods of management of the still cold extremity have included:

- Anticoagulants such as heparin.
- Low Molecular Weight Dextran
- Sympathectomy
- Vasodilators
- Slow rewarming
- Fast rewarming

ANIMAL MODELS OF FROSTBITE:

There is no good animal model of frostbite that will allow quantifiable evaluation of various methods of treatment. This comes as a surprise for it is

In contrast to the many eleborate physiologic animal studies on the effect generalized body hypothermia. The best was described in our laboratories by Sjostrom, Paton, Weatherly-White and Knize in 1964, involving immersion of the rabbit ear in dry ice and alcohol. (J. Surg. Res. 4:12, 1964). Because we found this model as originally described highly variable in the actual cold exposure and degree of hypothermia it was modified as follows (Figure 1).

Adult New Zealand White rabbits (body weight, 1500/2000 gms) were anesthetized with intravenous Sodium Pentobarbital and an intravenous Ringer's Lactate solution administered at 0.5 ml/min in the right ear.

Characteristically the ear became hyperemic for one to two days and then gradually began to develop gangrene starting at the tip. By the seventh day, a stabilized border of dry black gangrene was obvious starting at approximately 5 cm from the tip on each margin. The central portion of the ear also characteristically was gangrenous to approximately 5 cm.

On the seventh day, when the extent of the gangrene had stabilized, the animal was anesthetized and sacrificed. And a second cardboard template was traced along the profile of the non-gangrenous portion of the ear. Comparing the weights of the two cardboard templates prior to and following cold exposure provided a measurement of the percent of tissue loss resulting from the cold exposure.

In untreated animals (n=67), the percent of tissue loss was repeatedly within a standard error of 24 %. To be certain that there was no variation between animals possibly cold conditioned during various parts of the year, paired simultaneous controls were made throughout the experimental period.

Study groups to date are as illustrated in Figure 2.

In addition to controls in which infusion in the opposite ear was only Ringers Lactate, the following experiments were performed.

Group 1. Nitrogen Mustard (NM_2) to achieve neutropenia. (n=13)

Thirteen animals were given 1.75 mg/kg of body weight NM_2 72 hours prior to cold immersion. Immediately prior to cold immersion a leukocyte count was performed to confirm that the peripheral white blood count had fallen. Characteristically, the total white count was 800 to 1,000 of which less than 1% were polymorphonuclear cells in the circulating blood.

These animals with neutropenia tolerated the cold exposure so poorly that a statistically significant quantitation of tissue damage was impossible. Five died.

Group 2. Dimethylsulfoxide (DMSO) prior to freezing (n=19)

Because DMSO is a known hydroxyl ion scavenger, 4 gm/kg of DMSO in 24 ml of physiologic saline was started in the ear of 19 animals prior to cold immersion and continued for 3 hours at the rate of 8 ml per hour following cold exposure. The total dose of DMSO was 750 mg per hour to a 1500 gm animal.

Group 3. DMSO on Rewarming (n=10)

The DMSO infusion was begun immediately prior to rewarming and continued for 3 hours.

Group 4. Oral Allopurinol prior to cold exposure (n=9)

Because allopurinol is an inhibitor of xanthine oxidase which in turn is necessary for the production of superoxide, this drug was administered to determine whether it was protective. Nine animals were given 50 mg/kg of allopurinol by mouth daily x 3 prior to cold exposure.

Group 5. Allopurinol plus DMSO prior to cold exposure. (n=37)
Oral allopurinol was given for three days prior to cold exposure as in Group 4 but, in addition, DMSO was administered intravenously immediately prior to cold exposure (as in Group 2 above), 4 gm/kg DMSO in 24 ml of physiologic saline. The DMSO was continued for three hours following cold exposure as in Group 2 above.

Group 6. Allopurinol before cooling plus DMSO upon rewarming. (n=11)
Eleven animals were given oral allopurinol just before freeze and the intravenous DMSO started only on rewarming.

Group 7. Local DMSO at time of rewarming. (n=14)
Fourteen rabbits had their ears immersed in 100% DMSO during the rewarming.

Group 8. Nitrogen Mustard plus DMSO. (n=12)
Nitrogen Mustard as in Group 1 and DMSO as in Group 2.

Group 9. Dimethylthiourea (DMTU) (n=10)
A bolus of 500 mg/kg DMTU in 10 ml of saline was given intravenously 15 minutes before cold exposure. The infusion was maintained at 8 ml per hour and continued for three hours. This provided an infusion rate of 0.166 gms of DMTU per kg/hour.

Group 10. Allopurinol plus DMTU. (n=12)
Oral allopurinol as in Group 4 was combined with DMTU as in Group 9.

Group 11. Imidazole. (n=9)

Imidazole, a thromboxane synthetase inhibitor which is said to inhibit permeability edema, was administered as a 25 mg/kg per hour intravenous infusion starting 30 minutes prior to freeze and continued for one hour thereafter.

Group 12. Acetylcysteine. (n=11)

Acetylcysteine binds free radicals such as hydroxyl ion and was administered as a 150 mg/kg loading dose with a continuous intravenous infusion at 20 mg/kg/hour for three hours after rewarming. A final 100 mg/kg intravenous bolus was given prior to stopping the infusion.

Group 13. Intravenous Allopurinol. (n=10)

The sodium salt of allopurinol was administered as a 50 mg/kg intravenous bolus before freezing and one other 50 mg/kg intravenous bolus during the rewarming period. It was noted that this highly alkaline material required additional anesthesia in the animals presumably because it irritated the vein. To be certain that the additional anesthesia did not interfere with judging tissue loss from cold, the time required to reach $-5^{o}C$ was recorded in the animals receiving intravenous allopurinol, as well as in the controls. There was no difference in time of cold exposure.

Group 14. Allopurinol oral plus allopurinol intravenous and DMSO on rewarming. (n=23)

The intravenous allopurinol was given in doses as in Group 13,

DMSO as in Group 2, and oral allopurinol was given 2 days post freeze at a dose of 50 mg/kg.

Group 15. Colchicine. (n=8)

Colchicine inhibits mitosis in leukocytes and possibly inhibits chemotaxin. A bolus of 0.1 mg Colchicine was injected intravenous 15 minutes prior to freeze and another bolus of 0.1 mg Colchicine intravenous upon rewarming.

Group 16. Superoxide Dismutase (SOD). (n=5)

An intravenous bolus of SOD 2.5 mg/kg was administered just before freeze.

RESULTS:

As illustrated in Figure 2, the mean tissue loss is 78% with a standard deviation of 23% in the untreated controls.

There was no statistically valid alteration in tissue damage from cold by any of the following treatment regime.

DMSO prior to freee (n=19)

DMSO on rewarming (n=10)

Allopurinol oral, 3 days (n=9)

Allopurinol oral immediately prior to freeze and DMSO on rewarming (n=11)

DMSO local on rewarming (n=14)

Nitrogen Mustard (n=13)

Nitrogen Mustard plus DMSO prior to freeze (n=12)

DMTU before freeze (n=10)

Allopurinol 3 days plus DMTU prior to freeze (n=12)

Imidazole before freeze (n=9)

Acetylcysteine before freeze (n=11)

Allopurinol I.V. and DMSO prior to freeze (n=11)

Allopurinol I.V. and DMSO prior to freeze plus oral allopurinol 3 days after freeze (n=23)

Colchicine before freeze (n=8)

The two groups that had a statistically significant benefit were:

Group 5 Allopurinol oral 3 days prior to freeze plus DMSO started prior to freeze and continued for 3 hours after rewarming.

n = 37

Mean loss = 24%

Standard deviation = 22%

Group 13 Allopurinol intravenous one bolus prior to freeze and another

at time of rewarming.

n = 10

Mean loss = 19%

Standard Deviation = ± 33%

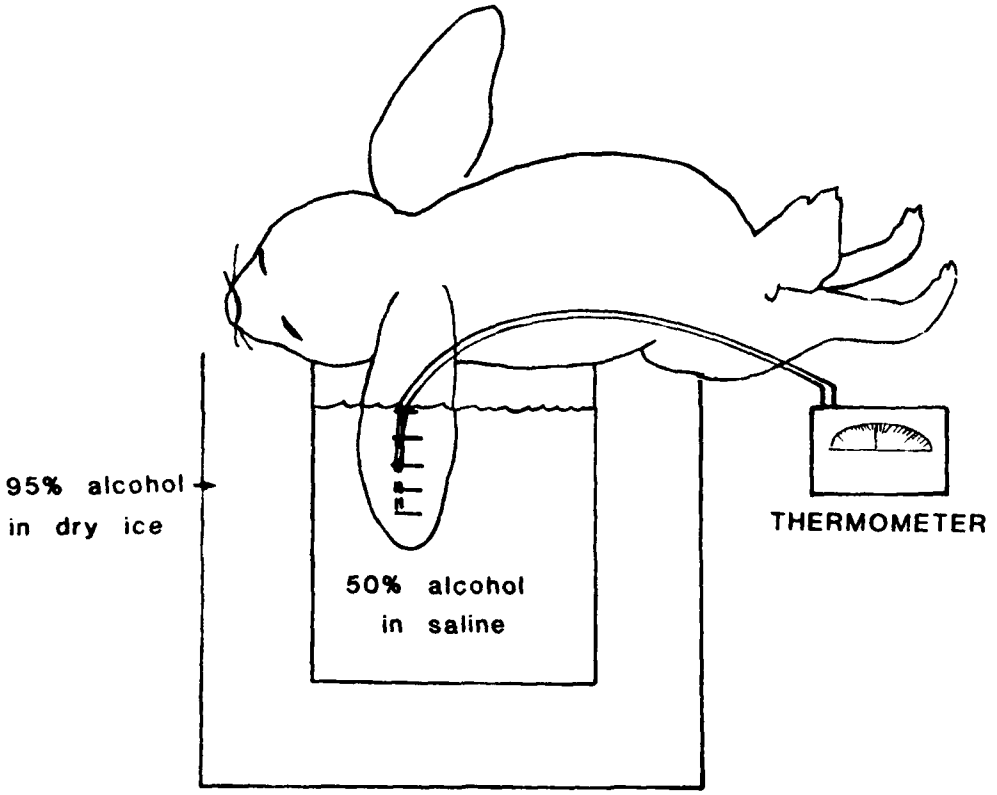
Although superoxide dismutase appears protective the number (n=5) are too small to be definitive.

We conclude that oral allopurinol with DMSO or intravenous allopurinol alone provides significant protection against cold injury.

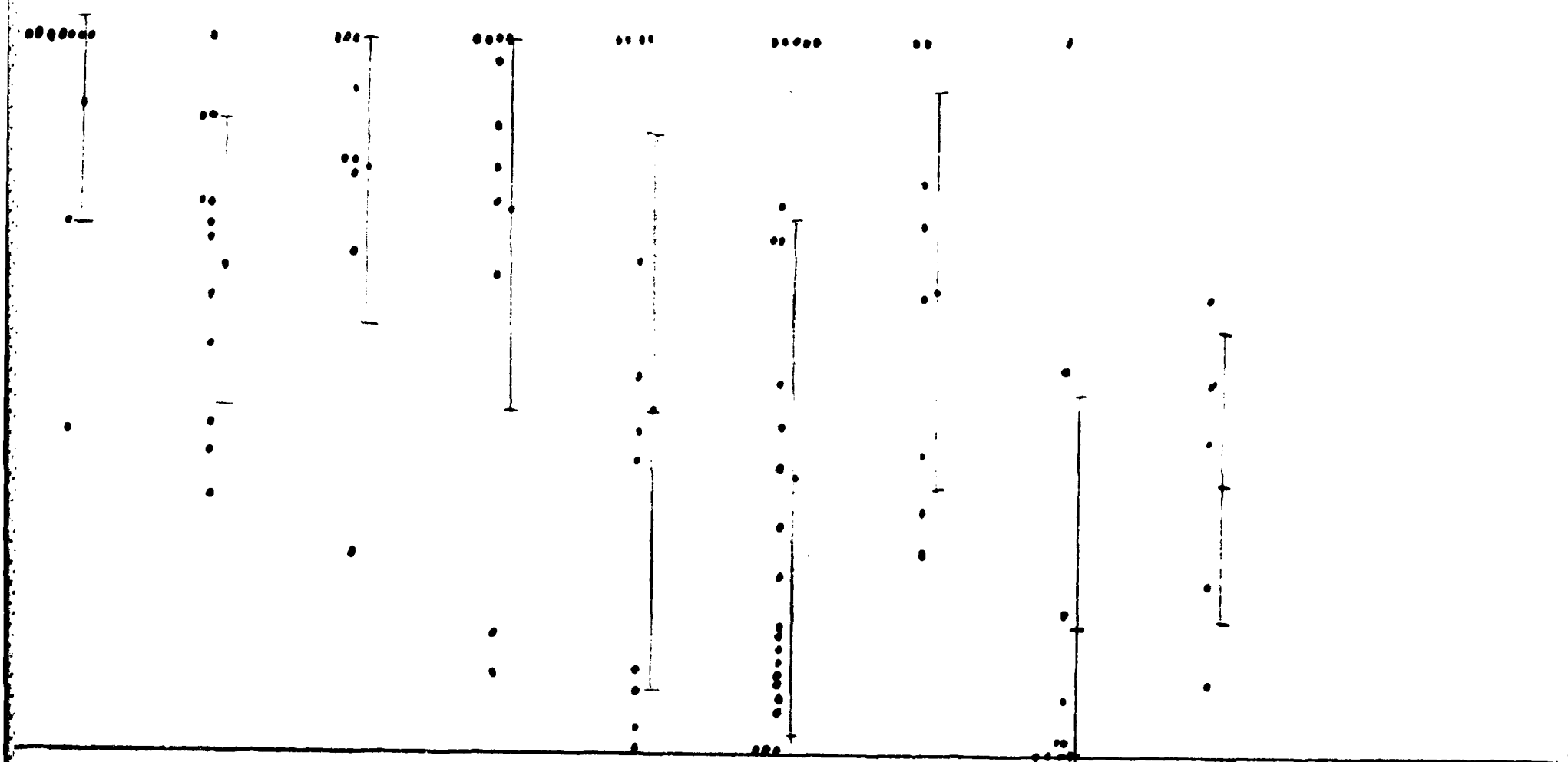
PROPOSED FUTURE EXPERIMENT:

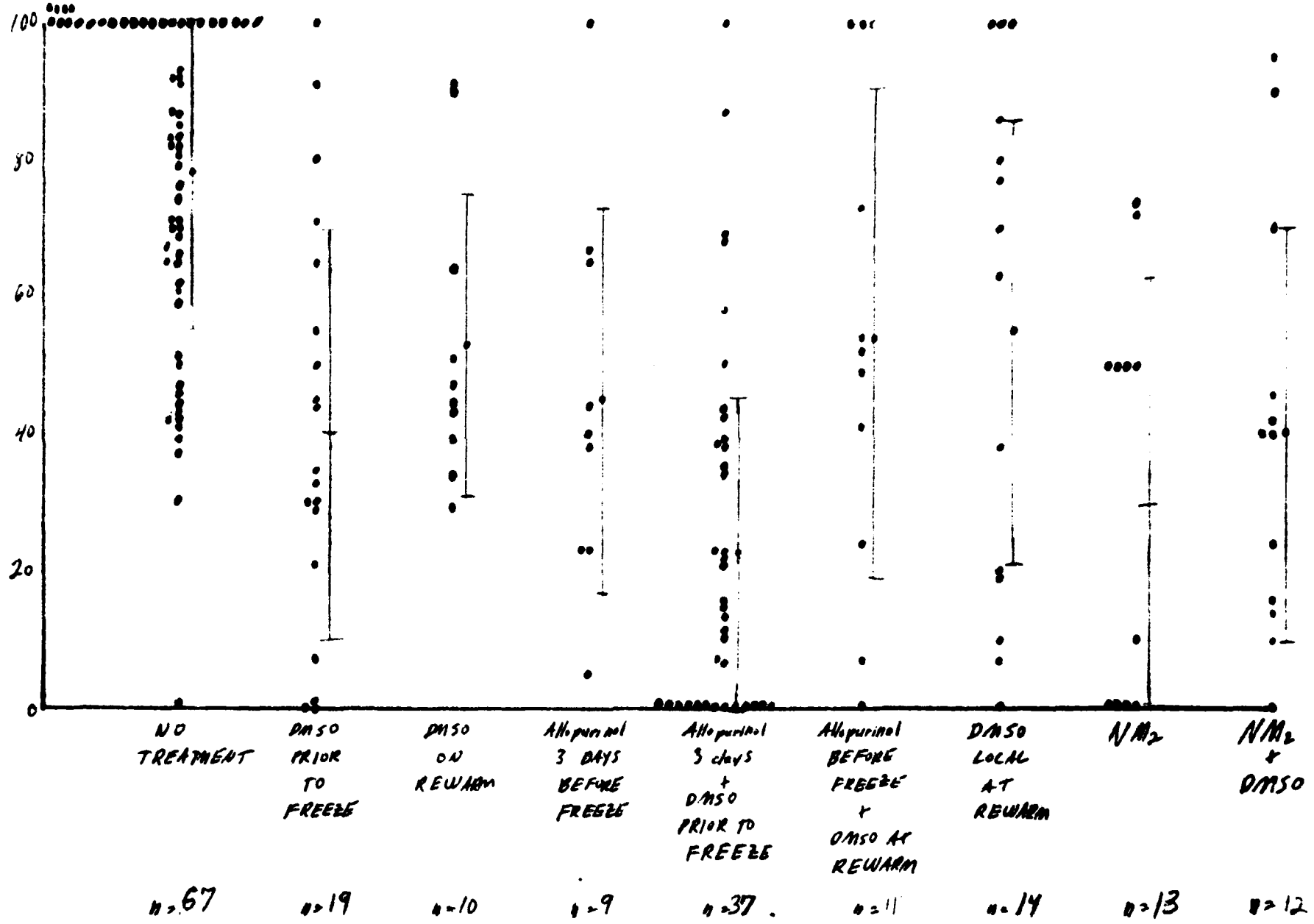
Having shown that drugs that alter superoxide production can afford protection against cold injury, we plan the following:

Group A	Superoxide Dismutase
Group B	Calcium blockers (Verapamil)
Group C	Cyclooxygenase inhibitor (Ibuprofen (Motrin)) or Indomethacine
Group D	Mannitol
Group E	Leukopenia produced by antileukocyte antibody



ANIMAL MODEL COLD INJURY


DMTU
Allopurinol + DMTU
Imidazole
acetyl cysteine
IV Allopurinol + DMSO
IV Allopurinol + DMSO + Allopurinol PO
Colchicine
IV Allopurinol
SOD
n=10
n=12
n=9
n=11
n=11
n=23
n=8
n=10
n=5


100
80
60
40
20
0
NO TREATMENT
DMSO PRIOR TO FREEZE
DMSO ON REWARM
Allopurinol 3 DAYS BEFORE FREEZE
Allopurinol 3 days + DMSO PRIOR TO FREEZE
Allopurinol BEFORE FREEZE + DMSO AT REWARM
DMSO LOCAL AT REWARM
NM2
NM2 + DMSO
n=67
n=19
n=10
n=9
n=37
n=11
n=14
n=13
n=12

[redacted]RJURY GRANT
[redacted]N00014-81-K0731

15 November 1982

BUDGET

YEAR 03

	% Time	Salary	Fringe Benefits	Total
PERSONNEL:				
Principle Investigator	10%	-0-	-0-	
Researcher II (Rebecca Sloan)	100%	$25,600	$4,096	$29,696
SUPPLIES:				
Rabbits @ $25/rabbit x 300 rabbits/year				$ 7,500
Rabbit Board @ 80¢/day x 300 x 10				2,400
Biologicals (dry ice, alcohol, DMSO, etc.)				5,500
Glassware, syringes, I.V.'s and disposables				2,400
Operating fees @ $17.50 x 300				5,250
				$23,050
TRAVEL:				
One meeting at $880				$ 880
MISCELLANEOUS:				
Duplication and books				$ 330
Pathology x $5 x 150				550
Medical illustrations				770
2 cold immersion chambers @ $250/each				500
2 Yellow Springs Temperature probes @ $200/each				400
				$ 2,550
			Direct costs	$56,176
			Indirect costs	$15,168
			TOTAL	$71,344

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